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(54) Title: A HUMAN CYTOMEGALOVIRUS COMBINED ANTIGEN AND ITS USE

(57) Abstract

A combined antigen having at least three portions of human cytomegalovirus (HCMV) proteins and characterized by an enhanced ability to bind HCMV-specific antibodies, for use in assays for the detection of HCMV-specific antibodies and as a vaccine to confer protective immunity against HCMV-mediated diseases.

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A HUMAN CYTOMEGALOVIRUS COMBINED ANTIGEN AND ITS USE

FIELD OF THE INVENTION

The present invention relates to the field of virology, specifically, human cytomegalovirus and the immune response to this infection.

BACKGROUND OF THE INVENTION

Human cytomegalovirus (HCMV) belongs to the herpes virus family. Infection with HCMV occurs frequently, as evidenced by the high percentage (over 50%) of adults having antibodies to this virus. Infection in the normal immunocompetent individual is mild or asymptomatic. However, in newborns and in the immunocompromised host such as organ and bone marrow transplant recipients and AIDS patients, severe disease develops (reviewed by Ho (1991) in: Cytomegalovirus: Biology and infection, (2nd ed.), Plenum Med. Press, New York).

Like other herpes viruses, HCMV can establish a life-long latency after initial infection (Stevens (1989) Microbiol. Rev. 53:318-332; Bruggeman (1993) Virchows Arch, B cell Pathol. 64:325-333). The site of latency is unknown. There are some data indicating that several organs and tissues such as kidney, heart and vessel wall of large vessels are sites of latency. In addition, blood cells such as macrophages can contain latent virus (Hendrix et al. (1989) Am. J. Pathol. 134:1151-1157; Yomashiroya et al. (1988) Am. J. Pathol. 130:71-79; Tanake et al. (1992) J. Vasc. Surg. 16:274-279; Stanier et al. (1989) Br. Med. J. 299:897-898; Bevan et al. (1991) Br. J. Haematol. 78:94-99; Taylor-Wiedeman et al. (1991) J. Gen. Virol. 72:2059-2064).

From the latent infection the virus can reactivat resulting in an endogenous infection posing a risk in the immunodeficient host. Both primary infections and reinfections (either endogenous, by reactivation of latent virus within the host or exogenous, by reinfection with a new virus from outside) can lead to acute (or active) infection. Especially primary infections can result in life-threatening disease (Rubin (1990) Rev. Infect. Dis. 12(suppl.7):S754-S766; Schooley (1990) Rev. Infect. Dis. 12(suppl.7):S811-S819).

Although the cellular immunity is the most important part of the immune response for clearing or reducing HCMV infection in the host, it is clear from studies in humans and in animal models that also humoral immunity has an effect on the course of the infection by reducing or preventing the CMV-associated symptoms (Meyers et al. (1983) Ann. Intern. Med. 98:442-446; Snijdman et al. (1987) New Engl. J. Med. 317:1049-1054; Stals et al. (1994) Antiviral Res. 25:147-160).

Recently, experiments in animal models have shown that clinical symptoms can be prevented by vaccination, supporting the finding that the presence of antibodies reduce CMV infection and, as a consequence, disease.

Although antiviral chemotherapy has been successful for some herpes viruses, especially for herpes simplex viruses, the prevention and treatment of HCMV infection remain difficult. The best results for HCMV therapy are obtained when the therapy is started very early in infection (Whitley & Gnann (1992) New Engl. J. Med. 327:782- 789; Meyers et al. (1988) New Engl. J. Med. 318:70-75; Collaborative DHPG treatment study group (1986) New Engl. J. Med. 314:801-806; Walmsley (1988) J. Infect. Dis. 157:569; Goodrich et al. (1991) New Engl. J. Med. 325:1601-1607;

Merigan et al. (1992) New Engl. J. Med. 326:1182-1186). Therefore, early detection of active HCMV infection is important. For the early detection of acute HCMV infection (either primary or reactivation of latent infection) th r is an increasing need for new specific and sensitive techniques. Besides the detection of virus, viral antigens and viral genome, detection of anti-HCMV antibodies, especially IgM (and to a lesser extent IgA) is important (Landini (1993) Prog. Med. Virol. 4:157-177; Bij vd W et al. (1988) J. Med. Virol. 25:179-188; Genna et al. (1991) J. Inf. Dis. 164:488-498; Nielsen et al. (1980) J. Clin. Microbiol. 26:654-661; Sarov et al. (1982) Clin. Exp. Immunol. 48:321-328).

The present invention addresses the need for early detection of HCMV by providing a synthetic protein useful in an assay for the early detection of anti-HCMV antibodies. The present invention further provides a HCMV vaccine.

BRIEF SUMMARY OF THE INVENTION

The invention features a human cytomegalovirus protein, also called a "combined antigen", having at least three HCMV protein epitopes, useful as a HCMV vaccine and in an assay for early detection of HCMV infection. The invention further features a method of preparing the combined antigen of the invention by recombinant DNA techniques.

In a specific embodiment, the combined antigen of the invention is a fusion protein having the amino acid sequence of SEQ ID NO:12. In this embodiment, the combined antigen is composed of six histidine residues and defined portions of the HCMV proteins UL32, UL83 and UL80. The type or the number of HCMV antigens included in the "combined" antigen is not limited, and may include more than three

epitopes. The antigens (epitopes) used in this assay show an enhanced ability to bind IgM, exhibiting a 2- to 3-fold increase in IgM antibody binding relative to a single antigen.

Included in the invention are nucleotide sequences which encode the combined antigen of the invention. Thes nucleotide sequences include DNA, cDNA and RNA sequences encoding the combined antigen of the invention. In a specific embodiment, the invention includes nucleotide sequences having the nucleotide sequence of SEQ ID NO:11. It is also understood that the nucleotide sequences of the invention include minor modifications of the nucleotide sequences encoding the combined antigen of the invention, so long as the resulting proteins have the same *in vitro* and/or *in vivo* activity and function of the protein encoded by the sequence of SEQ ID NO:11.

The invention further includes vectors containing the nucleotide sequences of the invention and host cells transformed with the vectors of the invention.

The present invention features an assay for detecting the presence and the amount of antibodies to HCMV-encoded antigens in tissue and biological fluid of infect d humans. This assay achieves improved sensitivity of immuno-detection by combining the immuno-dominant regions of early-formed proteins into a single protein. In addition, the combined antigen of the invention can be attached to a solid phase for use in a solid phase assay such as an immuno-assay or similar assays widely used for detecting both antigen and antibodies (IgG, IgM and IgA) in body samples. The enhanced ability of the combined antigen of the invention to bind HCMV-specific antibodies provides a sensitive assay able to detect HCMV-mediated diseases at an early stage of infection, thus allowing early treatment to commence.

In one aspect, the invention features use of the combined antigen as a human cytomegalovirus vaccine. Th combined antigen useful as a vaccine contains portions of the proteins encoded by HCMV sequences ppUL32, ppUL80 and ppUL83, made as described below. The vaccine of the invention is useful in conferring protective immunity in human subjects at risk for a HCMV-mediated disease.

These and other objects, advantages, and featur s of the invention will become apparent to those persons skilled in the art upon reading the details of the methods, assays, and peptides of the invention as more fully described below.

BRIEF DESCRIPTION OF THE DRAWING

Figs. 1A and 1B show the nucleic acid sequence and corresponding amino acid sequence of the exemplary combined antigen of the invention.

DETAILED DESCRIPTION

Before the present proteins, assays, and methods of use are described, it is to be understood that this invention is not limited to particular methods, assays, or proteins described, as such methods, assays and proteins may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present

invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

Combined Antigen

The present invention features a "combined antigen" having a portions of the amino acid sequences of the Towne strain of human cytomegalovirus. By the term "combined antigen" is meant a non-naturally occurring protein comprising in a single amino acid chain, all or an immunogenic part of the amino acid sequences of the proteins encoded by UL32 (ppUL32), UL80 (ppUL80), and UL83 (ppUL83). These amino acid sequences define epitopes which react efficiently with human immunoglobulins. The naturally occurring intact UL32 protein encodes a basic phosphoprotein of 150 kDa which binds serum from HCMV-infected patients. UL83 and UL80 encode the major HCMV matrix protein and assembly protein, respectively. The combined antigen protein of the invention binds HCMV-specific IgM with a 2- to 3-fold increased affinity relative to the naturally-occurring single epitope. The combined antigen of the invention has the amino acid sequence of SEQ ID NO:12.

By "enhanced ability to bind" or "increased binding affinity" is meant an improved binding of the combined antigen of the invention to HCMV-specific antibodies relative to a single epitope. Thus, the presence of the multiple epitopes in a single molecule provide a synergistic effect on binding to HCMV-specific antibodies. The terms "synergistic", "synergistic effect" and the like are used herein to describe improved binding to HCMV-specific antibodies of the combined antigen of the invention relative

to a single epitope. Although a synergistic effect in some fields means an effect which is more than additive (e.g., 1+1=3), in the medical field a synergistic effect may be additive (1+1=2) or less than additive (1+1=1.6). Thus, the presence of multiple antigenic domains in a single molecule is considered to provide a synergistic effect on HCMV-specific antibody binding (e.g., > 1.0) relative to a single domain (1.0).

The combined antigen of the invention is compris d of antigenic domains from proteins from the HCMV Towne strain which can efficiently detect anti-HCMV antibodies in biological samples. The combined antigen of the invention is further comprised of a 6 histidine residue tag used to purify the antigen. The histidine tag is not immunogenic and does not interfere with antibody detection.

The invention includes nucleotide sequences encoding the combined antigen of the invention. These nucleotide sequences can be expressed in either prokaryote or eukaryote host cells, including microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences are known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host ar known in the art. Such vectors are used to incorporate DNA sequences of the invention.

Transformation of a host cell with vectors containing DNA encoding the combined antigen of the invention may be carried out by conventional techniques as are well known to those skilled in the art. Such transformed host cells are capable of expressing the combined antigen. Isolation and purification of the expressed combined antigen may be carried out by conventional means well known in the art.

Assay Method for the Detection of HCMV Antibodies

The combined antigen of the present invention possesses advantages over prior art antigen preparations, including 2- to 3-fold improved binding to IgM antibodies. This improved IgM binding provides a more accurate and sensitive assay for the detection of HCMV antibodies present during early HCMV-mediated infection of a human subject.

By "HCMV-mediated infection" or "HCMV-mediated disease" is meant any pathological condition resulting from infection of a human with human cytomegalovirus, including congenital infections.

Those skilled in the development of immuno-reactive techniques will understand that there are numerous well known procedures for the detection of antibodies and uses of antigens for this purpose. Thus while only a few assay methods are described herein, the invention is not limited to those assays specifically described. Included in the detection assay of the invention are both competitive and non-competitive assay methods. Examples of assays methods in which the combined antigen of the invention can be used include radio-immuno-assay (RIA), western blotting, enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence (IF) assays.

For the detection of acute HCMV infections two approaches are possible. The first is based on the detection of virus or parts of it (antigens or genome). Although in general this approach gives good results, it needs specific equipment and knowledge and can usually only be applied in academic centers or large laboratories.

The second approach is based on the detection of IgM antibodies in serum of the patient and on a rise in IgG class antibodies. Detection of antibodies can easily be accomplished using techniques such as the ELISA technique.

In principle, this technique is relatively simple to handl and can be used in routine laboratories (Kraat et al. (1992) J. Clin. Microbiol. 30:522-524; Lazzaroto et al. (1992) J. Clin. Lab. Anal. 6:216-218; Stagno et al. (1985) J. Clin. Microbiol. 21:930-935; Smith & Shelley (1988) J. Virol. Meth. 21:87-96; Marsano et al. (1990) J. Inf. Dis. 161:454-461).

Although from a theoretical point of view ELISA is a simple technique for IgM antibody detection, there are a lot of problems associated with the use of commercial ELISA kits. Currently available CMV-IgM antibody detection methods suffer from considerably variations in specificity and sensitivity. This is largely due to differences in antigen composition and the lack of antigen standardization.

These problems are solved by combining three recombinant viral proteins (ppUL80 (p38), ppUL83 (pp65) and ppUL32 (ppl50)) into a single synthetic protein suitabl for detection of IgM antibodies. These viral proteins were employed to develop a sensitive method for early detection of acute HCMV infections in patients "at risk" such as organ recipients, premature infants and patients suffering from the acquired immunodeficiency syndrome (AIDS).

Human Cytomegalovirus Vaccine

Vaccination with inactivated or attenuated organisms or their products has been shown to be an effective method for increasing host resistance and ultimately has led to the eradication of certain common and serious infectious diseases. The use of vaccines is based on the stimulation of specific immune responses within a host.

The combined antigen described in this invention generates an immune response. The term "immune respons " refers to a cytotoxic T cell response or increased serum

levels of antibodies specific to an antigen, or to the presence of neutralizing antibodies to an antigen. The immune response is preferably sufficient to make the combined antigen of the invention useful as a vaccine for protecting human subjects from human cytomegalovirus infection. Additionally, antibodies generated by the combined antigen of the invention can be extracted and used to detect a virus in a body fluid sample. The term "protection" or "protective immunity" refers herein to th ability of the serum antibodies and cytotoxic T cell response induced during immunization to protect (partially or totally) against a disease caused by an infectious agent, e.g., human cytomegalovirus. The use of the combined antigen as a vaccine is expected to provide protective immunity to humans against severe HCMV infection by inducing antibodies against HCMV which are known to prevent severe clinical symptoms.

The invention includes a method of providing an immune response and protective immunity to a human against human cytomegalovirus-mediated diseases. The method includes administering the combined antigen of the invention to a human. The combined antigen of the invention is preferably administered as a formulation comprising a physiologically acceptable carrier and an effective amount of the combined antigen. A variety of physiologically acceptable carriers are known in the art, including for example, saline. Routes of administration, amounts, and frequency of administration are known to those skilled in the art for providing protective immunity to a recipient subject. Routes of administration include any method which confers protective immunity to the human recipient, including, but not limited to, inhalation, intravenous, intramuscular, intraperitoneal, intradermal, and

subcutaneous. Preferably the combined antigen of the invention is provided to a human subject by subcutaneous or intramuscular injection. A range of amounts and frequency of administration is acceptable so long as protective immunity of the recipient is achieved. For example, 5 to 20 μg can be administered by intramuscular injection between 2 to 4 times over a three month period.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a compl te disclosure and description of how to make and use the assays of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.) but some experimental error and deviations should be accounted for. Unless otherwise indicated, temperature is in degrees Centigrade, molecular weight is average molecular weight, and pressure is at or near atmospheric.

Example 1. Construction of a vector which expresses part of ppUL80 from HCMV (Towne strain) as a fusion with six histidines.

Bacterial strains. All DNA cloning studies wer done using *Escherichia coli* strain DHSα. Protein expression experiments were performed with *E. coli* BL21 (DE3) plysS.

Protein expression and purification. Bacteria were grown in TB medium containing ampicillin and chloramphenicol to an OD_{600} of 1.0, after which protein expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) at 0.1 mM. One-step affinity chromatography of 6

histidine (6H) fusion proteins over Ni2+-chelating sepharos (Probond, Invitrogen) was carried out essentially as described by the manufacturers of the column material. Immunoblotting and ELISA experiments were conducted using standard techniques.

DNA fragment. The DNA fragment that encodes part of the ppUL80 protein of HCMV (Towne strain) was generated by PCR amplification. To develop oligonucleotides for PCR, we first had to determine the DNA sequence of part of the UL80 gene of the Towne strain. To this purpose, two oligonucleotides were generated which are homologous to UL80 sequences of the AD169 strain of HCMV. These oligonucleotides are of the sequence:

5'-GGGT*GAATTC*CAGTTGGCGGCACGTCAC-3' (ppUL80-N-EI) (SEQ ID NO:1) and

5'-CGCG*GAATTC*TTTATTAGGGTATCACGGTAG-3' (ppUL80-C-EI) (SEQ ID NO:2).

The sequences in bold print are identical to HCMV AD169 nucleotides 116475 to 116493 for ppUL80-N-EI, and complementary to nucleotides 117363 to 117386 for ppUL80-C-EI. The sequences in italics represent a recognition site for restriction endonuclease *Eco*RI. The oligonucleotides were used in PCR (1 cycle: 5 min at 94°C; 30 cycles: 1 min at 94°C, 1 min at 55°C, 1 min at 72°C; 1 cycle: 10 min at 72°C) with DNA from the HCMV Towne strain as template. The resulting PCR product was cloned and sequenced. Based on this sequence, Towne strain-specific oligonucleotides were designed which were employed to amplify part of the Towne UL80 gene. To facilitate cloning, *Eco*RI restriction endonuclease cleavage sites were introduced in the DNA primers; these *Eco*RI sites are indicated below in italics. The sequences of the primers are:

5'-TGAGTGAATTCGCGGACTACGTGGATCCCC-3' (ppUL80-N2-EI) (SEQ ID NO:3) and

5'-AGCTTGAATTCCACCATGTCTTTGGGCGG-3' (ppUL80-C2-EI) (SEQ ID NO:4)

The nucleotides in bold print correspond to HCMV AD169 nucleotides 116497 to 116515 for ppUL80-N2-EI and nucleotides 117259 to 117278 for ppUL80-C2-EI. After amplification, the PCR product was purified, digested with *Eco*RI and cloned into the *Eco*RI site of vector pRSET B (Invitrogen). In the resulting plasmid, the UL80 gene fragment is present at the 3' end of and in-frame with a fragment encoding six histidines (6H).

Example 2. Construction of a vector which expresses part of ppUL83 from HCMV (Towne strain) as a fusion with 6 histidines.

The DNA fragment that encodes part of the ppUL83 protein of HCMV (Towne strain) was generated by PCR. Oligonucleotides were developed which are homologous to the sequence of the Towne UL82 gene (Pande et al. (1991) Virology 182:220-228). *Bam*HI restriction endonuclease cleavage sites were introduced in the DNA primers; these sites are indicated below in italics. The sequences of the primers are:

5'-CT*GGATCC*GGCTTTTACCTCACACG-3' (ppUL83-N-BI) (SEQ ID NO:5) and

5'-TG*GGATCC*CGTTGTCGGAATCCTCG-3' (ppUL83-C-BI) (SEQ ID NO:6)

The sequences in bold print of ppUL83-N-BI are identical to nucleotides 855 to 871 of the ppUL83 gene sequence. The bold sequence of ppUL83-C-BI is complementary to nucleotides 1380 to 1396 of the ppUL83 gene sequence. After PCR amplification, the PCR-product was purified,

digested with BamHI and cloned into the *Bgl*II site of vector pRSET C (Invitrogen). In the resulting plasmid, the UL82 gene fragment is present at the 3' end of and in-frame with a fragment encoding 6H.

Example 3. Construction of a vector which expresses part of ppUL32 from HCMV (Towne strain) as a fusion with 6H.

The DNA fragment that encodes part of the ppUL32 protein of HCMV (Towne strain) was generated by PCR, similarly as described for cloning of part of the UL80 gene (see above). Oligonucleotides for PCR were only developed after sequencing part of the UL32 gene of the Towne strain. To this purpose, two oligonucleotides were generated which are homologous to UL32 sequences of the AD169 strain of HCMV. The sequences of these oligonucleotides are:

5'-CGGTC*AAGCTT*CGTCGGTGTTCCTTCCTTG-3' (ppUL32-N-HIII) (SEQ ID NO:7) and

5'-CCGTC*AAGCTT*TCCCGACACGTCACTATCC-3' (ppUL32-C-HIII) (SEQ ID NO:8)

The sequences in italics represent *Hind*III cleavage sites. The sequences in bold print are complementary to HCMV AD169 nucleotides 40288 to 40306 for ppUL32-N-HIII, and identical to nucleotides 39783 to 39804 for ppUL32-C-HIII. PCR was carried out with DNA from the HCMV Towne strain as template. The resulting PCR product was cloned and sequenced. Based on this sequence, Towne strain-specific oligonucleotides were developed which were subsequently used to amplify part of the Towne UL32 gene. *Hind*III restriction endonuclease cleavage sites were introduced into the primers; these sites are shown in italics in the sequences below. The sequences of the primers are:

5'-TGGCA*AAGCTT*TGGTAGGTCGACCGCCCTC-3'

(ppUL32-N2-HIII) (SEQ ID NO:9) and

5'-TCGTCAAGCTTCCTCCGTGTTCTTAATCTTCTCG-3'
(ppUL32-C2-HIII)(SEQ ID NO:10)

The nucleotides in bold print correspond to HCMV AD169 nucleotides 40244 to 40262 for ppUL32-N2-HIII and nucleotides 39850 to 39874 for ppUL32-C2-HIII. After amplification, the PCR product was purified, cleaved with *Hind*III and cloned into the *Hind*III site of vector pRSET B (Invitrogen). In the resulting plasmid, the UL32 gene fragment is present at the 3' end of and in-frame with a fragment encoding 6 histidines.

Example 4. Construction of a vector which expresses parts of ppUL83, ppUL80 and ppUL32 from HCMV (Towne strain) as in-frame fusions with 6H.

To generate a plasmid which expresses a fusion protein of 6H and parts of ppUL83, ppUL80 and ppUL32, the DNA fragments encoding ppUL80 and ppUL32 were inserted into the *Eco*RI and *Hind*III sites, respectively, of the plasmid which contains the 6H-ppUL83 open reading frame (see Example 2 above). The resulting nucleic acid construct contains an in-frame fusion of the 6H-ppUL83 open reading frame and parts of the ppUL80 and ppUL32 genes which were described above. The amino acid sequence (SEQ ID NO:12) corresponding to the nucleic acid construct of the combined antigen (SEQ ID NO:11) of the invention are shown in Figs. 1A-1B.

Example 5. Sensitive Assay for HCMV Antibodies

The combined antigen can be used in an enzyme linked immunosorbent assay (ELISA) as an antigen adsorbed to a carrier solid phase or in a competition assay in which known specific antibodies compete with antibodies present in the patient's serum for the specific epitopes on the combined antigen. The combined antigen can also be conjugated to a

detection system, such as enzymes to detect serum antibodies which may be present in the patient's serum. An important advantage provided by the use of the combined antigen of th invention is that there are equal molar amounts of each of these three immunodominant antigens simultaneously present in the detection system, resulting in the improved sensitivity of the present assay.

SEQUENCE LISTING

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(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGTGAATTC CAGTTGGCGG CACGTCAC 28

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGCGGAATTC TTTATTAGGG TATCACGGTA G 31

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGAGTGAATT CGCGGACTAC GTGGATCCCC 30

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AGCTTGAATT CCACCATGTC TTTGGGCGG 29

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGGATCCGG CTTTTACCTC ACACG 25

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGGGATCCCG TTGTCGGAAT CCTCG 25

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGTCAAGCT TCGTCGGTGT TCCTTCCTTG 30

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCGTCAAGCT TTCCCGACAC GTCACTATCC 30

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGCAAAGCT TTGGTAGGTC GACCGCCCTC 30

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCGTCAAGCT TCCTCCGTGT TCTTAATCTT CTCG 34

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1896 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: cDNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGCGGGGTT CTCATCATCA TCATCATCAT GGTATGGCTA GCATGACTGG TGGACAGCAA 60
ATGGGTCGGG ATCTGTACGA CGATGACGAT AAGGATCGAT GGATCCGACC TCGAGATCCG 120
GCTTTTACCT CACACGAGCA TTTTGGGCTG CTGTGTCCCA AGAGCATCCC GGGCCTGAGC 180
ATCTCAGGTA ACCTATTGAT GAACGGGCAG CAGATCTTCC TGGAGGTGCA AGCGATACGC 240
GAGACCGTGG AACTGCGTCA GTACGATCCC GTGGCTGCGC TCTTCTTTTT CGATATCGAC 300
TTGCTGCTGC AGCGCGGGCC TCAGTACAGC GAACACCCCA CCTTCACCAG CCAGTATCGC 360
ATCCAGGGCA AGCTTGAGTA CCGACACACC TGGGACCGGC ACGACGAGGG TGCCGCCCAG 420
GGCGACGACG ACGTCTGGAC CAGCGGATCG GACTCCGACG AGGAACTCGT AACCACCGAG 480
CGCAAGACGC CCCGCGTTAC CGGCGGCGGC GCCATGGCGG GCGCCTCCAC TTCCGCGGGC 540
CGCAAACGCA AATCAGCATC CTCGGCGACG GCGTGCACGG CGGGCGTTAT GACACGCGGC 600
CGCCTTAAGG CCGAGTCCAC CGTCGCGCCC GAAGAGGACA CCGACGAGGA TTCCGACAAC 660
GGATCTGCAG CTGGTACCAT GGAATTCGCG GACTACGTGG ATCCCCATTA TCCCGGGTGG 720
GGTCGGCGTT ACGAGCCCGC GCCGTCTTTG CATCCGTCTT ATCCCGTGCC GCCGCCACCA 780
TCACCGGCCT ATTACCGTCG GCGCGACTCT CCGGGCGGTA TGGATGAACC ACCGTCCGGA 840
TGGGAGCGTT ACGACGGTAG TCACCGTGGT CAGTCGCAGA AGCAGCACCG TCACGGGGGC 900
AGCGGCGGAC ACAACAAACG CCGTAAGGAA GCCGCGGCGG CGTCGTCGTC CTCGGAGACA 960
GACTTGAGTT TCCCCGGCGA GGCCGAGCAC GGCCGGGCGC GAAAGCGTCT AAAAAGTCAC 1020
GTCAATAGCG ACGGTGGAAG TGGCGGGCAC GTGGGTTCCA ATCAGCAGCA GCAACAACGT 1080
TACGATGAAC TGCGGGATGC CATTCACGAG CTGAAACGCG ATCTGTTTGC TGCGCGGCAG 1140
AGTTCTACGT TACTTTCGGC GGCTCTTCCC GCTGCGGCCT CTTCCTCCCC GACTACTACT 1200
ACCGTGTGTA CTCCCACCGG CGAGCTGACG AGCGGCGGAG GAGAAACACC GACGGCACTT 1260
CTATCAGGAG GTGCCAAGGT AGCTGAGCGC GCTCAGGCCG GTGTGGTGAA CGCCAGTTGC 1320
CGCCTCGCTA CCGCGTCGGG TTCTGAGGCG GCAACGGCAG GGCCTTCGAC GGCGGGTTCT 1380
TCTTCCTGCC CGGCTAGTGT CGTGTTAGCC GCCGCTGCTG CCCAAGCCGC CGCAGCTTCC 1440
CAGAGCCCGC CCAAAGACAT GGTGGAATTC GAAGCTTTGG TAGGTCGACC GCCCTCGGTC 1500
CCCGTGAGCG GTAGCGCGCC GGGTCGCCTG TCCGGCACCA GCCGGGCCGC CTCGACCACG 1560
CCGACGTATC CCGCGGTAAC CACCGTTTAC CCACCGTCGT CTACGGCCAA AAGCAGCGTA 1620
TCGAATGCGC CGCCTGTGGC CTCCCCCTCC ATCCTGAAAC CGGGGGCGAG CGCGGCTTTG 1680
CAATCACGCC GCTCGACGGG GACCGCCGCC GTAGGTTCCC CCGTCAAGAG CACGACGGGC 1740
ATGAAAACGG TGGCTTTCGA CCTATCGTCG CCCCAGAAGA GCGGTACGGG GCCGCAACCG 1800
GGTTCTGCCG GCATGGGGGG CGCCAAAACG CCGTCGGACA CCGTGCAGAA CATCCTCCAA 1860
AAGATCGAGA AGATTAAGAA CACGGAGGAA GCTTGA 1896

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 631 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

1	M	R	G	S	H	H	H	H	H	H	G	M	A	S	M	T	G	G	Q	Q	20
21	M	G	R	D	L	Y	D	D	D	D	K	D	R	W	I	R	P	R	D	P	40
41	A	F	T	S	H	E	H	F	G	L	L	C	P	K	S	I	P	G	L	S	60
61	I	S	G	N	L	L	M	N	G	Q	Q	I	F	L	E	V	Q	A	I	R	80
81	E	T	V	E	L	R	Q	Y	D	P	V	A	A	L	F	F	F	D	I	D	100
101	L	L	L	Q	R	G	P	Q	Y	S	E	H	P	T	F	T	S	Q	Y	R	120
121	I	Q	G	K	L	E	Y	R	H	T	W	D	R	H	D	E	G	A	A	Q	140
141	G	D	D	D	V	W	T	S	G	S	D	S	D	E	E	L	V	T	T	E	160
161	R	K	T	P	R	V	T	G	G	G	A	M	A	G	A	S	T	S	A	G	180
181	R	K	R	K	S	A	S	S	A	T	A	C	T	A	G	V	M	T	R	G	200
201	R	L	K	A	E	S	T	V	A	P	E	E	D	T	D	E	D	S	D	N	220
221	G	S	A	A	G	T	M	E	F	A	D	Y	V	D	P	H	Y	P	G	W	240
241	G	R	R	Y	E	P	A	P	S	L	H	P	S	Y	P	V	P	P	P	P	260
261	S	P	A	Y	Y	R	R	R	D	S	P	G	G	M	D	E	P	P	S	G	280
281	W	E	R	Y	D	G	S	H	R	G	Q	S	Q	K	Q	H	R	H	G	G	300
301	S	G	G	H	N	K	R	R	K	E	A	A	A	A	S	S	S	S	E	T	320
321	D	L	S	F	P	G	E	A	E	H	G	R	A	R	K	R	L	K	S	H	340
341	V	N	S	D	G	G	S	G	G	H	V	G	S	N	Q	Q	Q	Q	Q	R	360
361	Y	D	E	L	R	D	A	I	H	E	L	K	R	D	L	F	A	A	R	Q	380
381	S	S	T	L	L	S	A	A	L	P	A	A	A	S	S	S	P	T	T	T	400
401	T	V	C	T	P	T	G	E	L	T	S	G	G	G	E	T	P	T	A	L	420
421	L	S	G	G	A	K	V	A	E	R	A	Q	A	G	V	V	N	A	S	C	440
441	R	L	A	T	A	S	G	S	E	A	A	T	A	G	P	S	T	A	G	S	460
461	S	S	C	P	A	S	V	V	L	A	A	A	A	A	Q	A	A	A	A	S	480
481	Q	S	P	P	K	D	M	V	E	F	E	A	L	V	G	R	P	P	S	V	500
501	P	V	S	G	S	A	P	G	R	L	S	G	T	S	R	A	A	S	T	T	520
521	P	T	Y	P	A	V	T	T	V	Y	P	P	S	S	T	A	K	S	S	V	540
541	S	N	A	P	P	V	A	S	P	S	I	L	K	P	G	A	S	A	A	L	560
561	Q	S	R	R	S	T	G	T	A	A	V	G	S	P	V	K	S	T	T	G	580
581	M	K	T	V	A	F	D	L	S	S	P	Q	K	S	G	T	G	P	Q	P	600
601	G	S	A	G	M	G	G	A	K	T	P	S	D	T	V	Q	N	I	L	Q	620
621	K	I	E	K	I	K	N	T	E	E	A	*									

What is claimed is:

1. A combined antigen having a portion of the amino acid sequence of at least three human cytomegalovirus (HCMV) proteins and characterized by an enhanced ability to bind human cytomegalovirus-specific antibodies.

2. The combined antigen of claim 1, further comprising six histidine residues.

3. The combined antigen of claim 1, wherein said antigen comprises portions of HCMV proteins encoded by UL80, UL83, and UL32.

4. The combined antigen of claim 1, wherein said antigen is encoded by the nucleic acid sequence formed by the sequence of SEQ ID NO:11.

5. The combined antigen of claim 1, wherein said HCMV-specific antibodies are IgM antibodies.

6. The combined antigen of claim 1, wherein said antigen has a 2- to 3-fold enhanced ability to bind HCMV-specific antibodies.

7. An assay device, comprising:

a support surface; and

a combined antigen bound to said surface, wherein the combined antigen comprises a portion of the amino acid sequence of at least three human cytomegalovirus (HCMV) proteins characterized by an enhanced ability to bind HCMV-specific antibodies.

8. A method for detecting and quantifying human cytomegalovirus (HCMV)-specific antibodies in a sample of human body fluid or tissue, said method comprising:

a) obtaining a sample of human body fluid or tissue;

b) contacting said sample with a combined antigen, wherein said protein contains a portion of the amino acid sequence of at least three human cytomegalovirus (HCMV) proteins and characterized by an enhanced ability to bind human cytomegalovirus-specific antibodies;

c) detecting the amount of combined antigen bound to HCMV-specific antibodies, wherein the amount of bound combined antigen is indicative of the presence of HCMV-specific antibodies.

9. The method of claim 8, wherein said combined antigen is labelled.

10. A vaccine for conferring protective immunity against human cytomegalovirus-mediated diseases, said vaccine comprised of the combined antigen of claim 1.

Fig. 1A : DNA and amin acid sequence of the combined antigen.

```
   1  ATGCGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAA   60
   1   M  R  G  S  H  H  H  H  H  H  G  M  A  S  M  T  G  G  Q  Q    20

  61  ATGGGTCGCGGATCTGTACGACGATGACGATAAGGATCGATGGATCCGACCTCGAGATCCG  120
  21   M  G  R  D  L  Y  D  D  D  D  K  D  R  W  I  R  P  R  D  P    40

      {pp65 ->
 121  GCTTTTACCTCACACGAGCATTTTGGGCTGCTGTGTCCCAAGAGCATCCCCGGCCTGAGC  180
  41   A  F  T  S  H  E  H  F  G  L  L  C  P  K  S  I  P  G  L  S    60

 181  ATCTCAGGTAACCTATTGATGAACGGGCAGCAGATCTTCCTGGAGGTGCAAGCGATACGC  240
  61   I  S  G  N  L  L  M  N  G  Q  Q  I  F  L  E  V  Q  A  I  R    80

 241  GAGACCGTGGAACTGCGTCAGTACGATCCCGTGGCTGCGCTCTTCTTTTTCGATATCGAC  300
  81   E  T  V  E  L  R  Q  Y  D  P  V  A  A  L  F  F  F  D  I  D   100

 301  TTGCTGCTGCAGCGCGGGCCTCAGTACAGCGAACACCCCACCTTCACCAGCCAGTATCGC  360
 101   L  L  L  Q  R  G  P  Q  Y  S  E  H  P  T  F  T  S  Q  Y  R   120

 361  ATCCAGGGCAAGCTTGAGTACCGACACACCTGGGACCGGCACGACGAGGGTGCCGCCCAG  420
 121   I  Q  G  K  L  E  Y  R  H  T  W  D  R  H  D  E  G  A  A  Q   140

 421  GGCGACGACGACGTCTGGACCAGCGGATCGGACTCCGACGAGGAACTCGTAACCACCCAG  480
 141   G  D  D  D  V  W  T  S  G  S  D  S  D  E  E  L  V  T  T  E   160

 481  CGCAAGACGCCCCCGCGTTACCGGCGGCGGCGCCATGGCGGGCGCCTCCACTTCCGCGGC  540
 161   R  K  T  P  R  V  T  G  G  G  A  M  A  G  A  S  T  S  A  G   180

 541  CGCAAACGCAAATCAGCATCCTCGGCGACGGCGTGCACGGCGGGCGGTTATGACACGCGGC  600
 181   R  K  R  R  S  A  S  S  A  T  A  C  T  A  G  V  M  T  R  G   200

                                                          <- pp65}
 601  CGCCTTAAGGCCGAGTCCACCGTCGCGCCCGAAGAGGACACCGACGAGGATTCCGACAAC  660
 201   R  L  K  A  E  S  T  V  A  P  E  E  D  T  D  E  D  S  D  N   220

                                    {p38 ->
 661  GGATCTGCAGCTGGTACCATGGAATTCGCGGACTACGTGGATCCCCATTATCCCGGGTCG  720
 221   G  S  A  A  G  T  M  E  F  A  D  Y  V  D  P  H  Y  P  G  W   240

 721  GGTCGGCGTTACGAGCCCGCGCCGTCTTTGCATCCGTCTTATCCCGTGCCGCCGCCACCA  780
 241   G  R  R  Y  E  P  A  P  S  L  H  P  S  Y  P  V  P  P  P  P   260

 781  TCACCGGCCTATTACCGTCGGCGCGACTCTCCGGGCGGTATGGATGAACCACCGTCCGGA  840
 261   S  P  A  Y  Y  R  R  R  D  S  P  G  G  M  D  E  P  P  S  G   280

 841  TGGGAGCGTTACGACGGTAGTCACCGTGGTCAGTCGCAGAAGCAGCACCGTCACGGGGGC  900
 281   W  E  R  Y  D  G  S  H  R  G  Q  S  Q  K  Q  H  R  H  G  G   300

 901  AGCGGCGGACACAACAAACGCCGTAAGGAAGCCGCGGCGGCGTCGTCGTCCTCGGAGACA  960
 301   S  G  G  H  N  K  R  R  K  E  A  A  A  A  S  S  S  S  E  T   320

 961  GACTTGAGTTTCCCCGGCGAGGCCGAGCACGGCGGGCGCGAAAGCGTCTAAAAAGTCAC 1020
 321   D  L  S  F  P  G  E  A  E  H  G  R  A  R  K  R  L  K  S  H   340

1021  GTCAATAGCGACGGTGGAAGTGGCGGGCACGTGGGTTCCAATCAGCAGCAGCAACAACGT 1080
 341   V  N  S  D  G  G  S  G  G  H  V  G  S  N  Q  Q  Q  Q  Q  R   360

1081  TACGATGAACTGCGGGATGCCATTCACGAGCTGAAACGCGATCTGTTTGCTGCGCGGCAG 1140
 361   Y  D  E  L  R  D  A  I  H  E  L  K  R  D  L  F  A  A  R  Q   380
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FIG. 1B

```
1141  AGTTCTACGTTACTTTCGGCGGCTCTTCCCGCTGCGGCCTCTTCCTCCCCGACTACTACT  1200
 381   S  S  T  L  L  S  A  A  L  P  A  A  A  S  S  S  P  T  T  T   400

1201  ACCGTGTGTACTCCCACCGGCGAGCTGACGAGCGGCGGAGGAGAAACACCGACGGCACTT  1260
 401   T  V  C  T  P  T  G  E  L  T  S  G  G  G  E  T  P  T  A  L   420

1261  CTATCAGGAGGTGCCAAGGTAGCTGAGCGCGCTCAGGCCGGTGTGGTGAACGCCAGTTGC  1320
 421   L  S  G  G  A  K  V  A  E  R  A  Q  A  G  V  V  N  A  S  C   440

1321  CGCCTCGCTACCGCGTCGGGTTCTGAGGCGGCAACGGCAGGGCCTTCGACCGCGGGTTCT  1380
 441   R  L  A  T  A  S  G  S  E  A  A  T  A  G  P  S  T  A  G  S   460

1381  TCTTCCTGCCCCGCTAGTGTCGTGTTAGCCGCCGCTGCTGCCCAAGCCGCCGCAGCTTCC  1440
 461   S  S  C  P  A  S  V  V  L  A  A  A  A  A  Q  A  A  A  A  S   480

                          <- p38]          [pp150 ->
1441  CAGAGCCCGCCCAAAGACATGGTGGAATTCGAAGCTTTGGTAGGTCGACCGCCCTCGGTC  1500
 481   Q  S  P  P  K  D  M  V  E  F  E  A  L  V  G  R  P  P  S  V   500

1501  CCCGTGAGCGGTAGCGCGCCGGGTCGCCTGTCCGGCACCAGCCGGGCCGCCTCGACCACG  1560
 501   P  V  S  G  S  A  P  G  R  L  S  G  T  S  R  A  A  S  T  T   520

1561  CCGACGTATCCCGCGGTAACCACCGTTTACCCACCGTCGTCTACGGCCAAAAGCAGCGTA  1620
 521   P  T  Y  P  A  V  T  T  V  Y  P  P  S  S  T  A  K  S  S  V   540

1621  TCGAATGCGCCGCCTGTGGCCTCCCCCTCCATCCTGAAACCGGGGGCGAGCGCGGCTTTG  1680
 541   S  M  A  P  P  V  A  S  P  S  I  L  K  P  G  A  S  A  A  L   560

1681  CAATCACGCCGCTCGACGGGGACCGCCGCCGTAGGTTCCCCCGTCAAGAGCACGACGGGC  1740
 561   Q  S  R  R  S  T  G  T  A  A  V  G  S  P  V  K  S  T  T  G   580

1741  ATGAAAACGGTGGCTTTCGACCTATCGTCGCCCCAGAAGAGCGGTACGGGGCGCCAACCG  1800
 581   M  K  T  V  A  F  D  L  S  S  P  Q  K  S  G  T  G  P  Q  P   600

1801  GGTTCTGCCGGCATGGGGGGCGCCAAAACGCCGTCGGACACCGTGCAGAACATCCTCCAA  1860
 601   G  S  A  G  M  G  G  A  K  T  P  S  D  T  V  Q  N  I  L  Q   620

                          <- pp150]
1861  AAGATCGAGAAGATTAAGAACACGGAGGAAGCTTGA
 621   K  I  E  K  I  K  N  T  E  E  A  *
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(57) Abstract

A combined antigen having at least three portions of human cytomegalovirus (HCMV) proteins and characterized by an enhanced ability to bind HCMV-specific antibodies, for use in assays for the detection of HCMV-specific antibodies and as a vaccine to confer protective immunity against HCMV-mediated diseases.

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 01321 A (ABBOTT LAB ;LANDINI MARIA PAOLA (IT); RIPALTI ALESSANDRO (IT); MAI) 18 January 1996 see the whole document ---	1-10
X	JOURNAL OF CLINICAL MICROBIOLOGY, vol. 33, no. 10, October 1995, pages 2535-2542, XP000608653 LANDINI M P ET AL: "RECOMBINANT MONO- AND POLYANTIGENS TO DETECT CYTOMEGALOVIRUS-SPECIFIC IMMUNOGLOBULIN M IN HUMAN SERA BY ENZYME IMMUNOASSAY" see the whole document ---	1-10
X	DE 44 26 453 C (BIOTEST AG) 2 November 1995 see the whole document --- -/--	1,2,5-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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8 August 1997	18. 08. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Mandl, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICAL ABSTRACTS, vol. 98, Philadelphia, PA, US; abstract no. 312163, RUAN Q ET AL: "Construction of a expression clone of an epitope gene of human cytomegalovirus by mutagenesis." XP002037112 see abstract & ZHONGHUA WEISHENGQUXUE HE MIANYIXUE ZAZHI 14 (5). 1994. 314-316. ISSN: 0254-5101, ---	1,2,5-10
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A	CURRENT TOPICS IN MICROBIOLOGY AND IMMUNOLOGY, vol. 154, 1990, pages 125-169, XP000611455 CHEE M S ET AL: "ANALYSIS OF THE PROTEIN-CODING CONTENT OF THE SEQUENCE OF HUMAN CYTOMEGALOVIRUS STRAIN AD169" see the whole document -----	1-10

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INTERNATIONAL SEARCH REPORT

...formation on patent family members

Int...nal Application No
PCT/EP 97/00865

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